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Carvedilol Analogue Modulates both Basal and Stimulated Sinoatrial Node Automaticity

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Abstract

Background—The membrane voltage clock and calcium (Ca^{2+}) clock jointly regulate sinoatrial node (SAN) automaticity. VK-II-36 is a novel carvedilol analog that suppress sarcoplasmic reticulum (SR) Ca^{2+} release but does not block β-receptor. The effect of VK-II-36 on SAN function remains unclear. The purpose of this study was to evaluate whether VK-II-36 can influence SAN automaticity through inhibiting the Ca^{2+} clock.

Methods and Results—We simultaneously mapped intracellular Ca^{2+} and membrane potential in 24 isolated canine right atriums, using previously described criteria of the timing of late diastolic intracellular Ca elevation (LDCAE) relative to the action potential upstroke to detect the Ca^{2+} clock. Pharmacological intervention with isoproterenol (ISO), ryanodine, caffeine, and VK-II-36 were performed after baseline recordings. VK-II-36 caused sinus rate downregulation and reduced LDCAE in the pacemaking site under basal condition ($P<0.01$). ISO induced an upward shift of the pacemaking site in SAN and augmented LDCAE in pacemaking site. ISO also significantly and dose-dependently increased the sinus rate. The treatment of VK-II-36 (30 μmol/L) abolished both the ISO-induced shift of pacemaking site and augmentation of LDCAE ($P<0.01$), and suppressed the ISO-induced increase in sinus rate ($P=0.02$).

Conclusions—Our results suggest that sinus rate may be partly controlled by Ca^{2+} clock via SR Ca^{2+} release during β-adrenergic stimulation.

Keywords

calcium; sympathetic nervous system; sinoatrial node; sarcoplasmic reticulum; store-overload-induced Ca release

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Conflict of Interest: None

Disclosures
None

Introduction

The mechanism of spontaneous diastolic depolarization (DD) of sinoatrial node (SAN) cells has traditionally been attributed to a membrane voltage clock mechanism, mediated by voltage-sensitive membrane currents, such as the hyperpolarization-activated pacemaker current (I_f) regulated by cAMP [1,2]. It was reported that ivabradine, a selective I_f current inhibitor, reduces spontaneous heart rate and has cardioprotective effects [3]. Some more recent studies implicate a complementary "Ca^{2+} clock" mechanism mediated by Ca^{2+} release from the sarcoplasmic reticulum (SR) causing DD via activation of Na/Ca exchanger current (I_{NCX}), which coordinately regulates sinus rate along with the membrane clock [4–6]. Recently, Himeno et al. [7] provided mathematical modeling and guinea pig single cell data supporting the predominance of the membrane clock in regulating SAN automaticity. Therefore, it remains unclear whether or not Ca^{2+} clock plays an important role in controlling sinus rate.

Ca^{2+} can be released from the SR through activation of the ryanodine receptor (RyR). Under normal conditions, the L-type Ca^{2+} channels are activated, leading to a small Ca^{2+} influx. This Ca^{2+} influx then activates RyRs, resulting in a large Ca^{2+} release from the SR and subsequent muscle contraction. The process is known as calcium-induced calcium release (CICR) [8]. In addition to this depolarization-stimulated Ca^{2+} release, Ca^{2+} can also be released spontaneously due to Ca^{2+} overload in the SR. When SR Ca^{2+} content reaches a critical level, spontaneous SR Ca^{2+} release in the form of Ca^{2+} waves or Ca^{2+} oscillations occurs in cardiac cells in the absence of membrane depolarization [9,10]. Under conditions of SR Ca^{2+} overload by a variety of factors such as catecholamine or stresses, spontaneous SR Ca^{2+} release occurs as a result of RyRs activation by SR luminal Ca^{2+} [11]. Jiang et al. [12] referred this depolarization-independent Ca^{2+} overload-induced SR Ca^{2+} release as store-overload-induced Ca release (SOICR). Furthermore, they reported that RyR mutations in catecholaminergic polymorphic ventricular tachycardia (CPVT) reduce the threshold for SOICR by increasing the sensitivity of the channel to activation by luminal Ca^{2+}, and enhancing the propensity for delayed afterdepolarizations and triggered arrhythmias under conditions of SR Ca^{2+} overload [13]. CPVT is typically associated with sinus bradycardia [14,15]. We reported in previous publications that spontaneous SR Ca release is important in the SAN automaticity during β-adrenergic stimulation, and it acts synergistically with activation of membrane ionic currents such as I_f to accelerate the sinus rate in intact canine SAN [16–19].

Recently, Zhou et al. [20] demonstrated that carvedilol suppressed SOICR independently of its β-blocking effect and prevented CPVT in RyR mutant mice. VK-II-36 is a carvedilol analog that does not significantly block β receptor. We demonstrated that VK-II-36 could suppress ventricular arrhythmias by inhibiting triggered activities [21]. Because SAN activity may share mechanisms underlying both automaticity and triggered activity [22], we hypothesize that SAN treated with VK-II-36 could reduce spontaneous SR Ca release, leading to suppressed SAN automaticity. In this study, we performed dual optical mapping of transmembrane potential (V_m) and intracellular Ca^{2+} (Ca_i) with intact canine RAs. We

studied the effects of VK-II-36 on sinus rate at baseline and during β-adrenergic stimulation to test that hypothesis.

Materials and Methods

Langendorff-perfused canine SAN preparation

This study protocol was approved by the Institutional Animal Care and Use Committee of Indiana University School of Medicine and the Methodist Research Institute, and conforms to the guidelines of the American Heart Association. We studied isolated canine RAs in 24 mongrel dogs (22 to 28 kg). The heart was rapidly excised under general anesthesia and the right coronary artery was perfused with 37°C Tyrode's solution equilibrated with 95% O_2 and 5% CO_2 to maintain a pH of 7.4. The composition of Tyrode's solution was (in mmol/L): 125 NaCl, 4.5 KCl, 0.25 $MgCl_2$, 24 $NaHCO_3$, 1.8 NaH_2PO_4, 1.8 $CaCl_2$, and 5.5 glucose). The coronary perfusion pressure was regulated between 50 and 60 mmHg. To ensure adequate atrial perfusion, all ventricular coronary branches were tied off. Both ventricles and left atrium were removed. Because SAN is subepicardial in dogs [24], we mapped the epicardial side of the tissue. The SAN area was typically located posterior to the sulcus terminalis. Contractility was inhibited by 10 – 17 μmol/L of blebbistatin, and the motion artifact was negligible even after isoproterenol (ISO) infusion. Pseudo-ECG was recorded with widely spaced bipolar RA electrodes using ISO-DAM8A (World precision instruments, FL, USA).

Dual V_m and Ca_i recordings

Optical mapping analysis was performed as previously described [25]. The hearts were stained with Rhod-2 AM and RH237 (Molecular Probes) and excited with laser light at 532 nm. Fluorescence was collected using 2 cameras (MiCAM Ultima, BrainVision, Tokyo, Japan) at 1 ms/frame and 100 × 100 pixels with spatial resolution of 0.35 × 0.35 mm^2/pixel. After mapping baseline spontaneous beats, pharmacologic intervention was performed in 24 isolated canine RAs. At first, the VK-II-36 dose response (1 to 30 μmol/L) was evaluated on SAN and surrounding RA (n=5). Next, we determined the ISO dose response (0.01 to 1.0 μmol/L) of SAN function under basal condition (n=5), and examined the response against ISO during 30 μmol/L VK-II-36 infusion (n=4). Furthermore, we examined the effects of ryanodine, RyR inhibitor, to SAN function. In 4 dogs, the ryanodine dose response (0.1 to 10 μmol/L) of sinus rate was evaluated. In the same dogs, we also determined the ISO dose response of sinus rate during 3 μmol/L ryanodine infusion. To identify effects of caffeine on SAN automaticity, which triggers Ca^{2+} release by activating RyR, caffeine (20 mmol/L, 2 mL) was given as a bolus injection into the coronary artery within 1 second (n=3). In 3 dogs, we examined whether or not the pretreatment of VK-II-36 (30 μmol/L) influences against the effects of caffeine on SAN automaticity. The synthesis of VK-II-36 is described in our previous paper [21].

Data Analysis

Sinus rate was defined as the rate generated by SAN activations confirmed with optical mapping. The Ca_i and V_m traces were normalized to their respective peak-to-peak amplitude for comparison of timing and morphology. The slopes of late diastolic intracellular Ca

elevation (LDCAE) were measured from the onsets of LDCAE and to peak levels of LDCAE. The onsets of LDCAE were defined by the time of the transition between negative to positive values in dCa_i/dt curves [16]. Student's t-tests were used to compare means between two groups. One-way analyses of variance followed by Bonferroni-Dunn test were used to compare three or more groups. Data were presented as mean ± SEM. A P-value of <0.05 was considered statistically significant.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

Effect of a SOICR blocker, VK-II-36, on SAN function

The averaged basal sinus rate of intact canine SAN used in the present study was 101±4 bpm (range 82 – 132 bpm, n=24). Figure 1 shows changes in sinus rate after the treatment of VK-II-36 or ryanodine. Both VK-II-36 and ryanodine significantly and dose-dependently decreased sinus rate. Figure 2 shows the change of activation pattern in SAN and the surrounding RA by VK-II-36. Figure 2b represents an example of the activation pattern on SAN and surrounding RA during spontaneous sinus rhythm. Under basal conditions, the pacemaking sites were located in the middle SAN. The upstrokes of Ca_i and V_m were nearly simultaneous. Small amplitude LDCAEs in pacemaking site were observed in the middle SAN at baseline recording in all preparations (arrows in Figure 2b, range 0.82 – 2.49 AU/s, n=24). The treatment of VK-II-36, which blocks SOICR, resulted in a downward (caudal) shift of the pacemaking site to lower SAN (Figure 2c, n=5). VK-II-36 infusion made LDCAE disappearance in the pacemaking site (dotted line arrow in Figure 2c). Figure 2d shows that VK-II-36 reduced LDCAE dose-dependently ($P<0.01$).

Effects of VK-II-36 on SAN function in the presence of β-adrenergic stimulation

ISO infusion significantly increased sinus rate, while the pretreatment of VK-II-36 or ryanodine reduced this ISO effect (Figure 3a). ISO (1.0 μmol/L)-induced increase in heart rate was significantly suppressed by VK-II-36 (30 μmol/L, P=0.02) or ryanodine (3 μmol/L, P=0.007) (Figure 3b). Figure 4 shows the effect of VK-II-36 on ISO-induced activation pattern change. ISO infusion resulted in an upward (cranial) shift of the pacemaking site (Figure 4a), coincident with the appearance of robust LDCAEs (arrows in Figure 4a). Figure 4b shows the impact of β-adrenergic stimulation on the SAN function after 30 μmol/L VK-II-36 treatment. VK-II-36 treatment inhibited ISO-induced upward shifting of the pacemaking site and the augmentation of LDCAE (Figure 4b). Figure 4c shows the distance of upward shifting of the pacemaking site dose-dependently when ISO infusion was increased from 0.01 to 1.0 μmol/L. VK-II-36 pretreatment almost completely inhibited ISO-induced upward shifting of the leading pacemaker site (ISO 1.0 μmol/L: 5.9 ± 0.3 (VK-, n=5) vs. 0.8 ± 0.1 mm (VK+, n=4), $P<0.01$, Figure 4c). ISO also dose-dependently augmented LDCAE. When ISO infusion was 1.0 μmol/L, the slope of LDCAE increased to 4.5±0.3 AU/s. VK-II-36 treatment significantly inhibited ISO-induced augmentation of LDCAE (1.0±0.2 AU/s, $P<0.01$, Figure 4d).

Effects of VK-II-36 on caffeine-induced changes in sinus node automaticity

Caffeine sensitizes RyRs to activation, resulting in increased SR Ca^{2+} release. We examined whether or not the treatment of VK-II-36 (30 μmol/L) suppress the effects of caffeine on SAN automaticity (Figure 5). When a 2-mL caffeine bolus (20 mmol/L) was injected directly into the right coronary artery, the pacemaking site was soon shifted to upward, and sinus rate increased from 95.0 ± 8.5 to 158.0 ± 5.0 bpm (P=0.001). Caffeine enhanced the slope of LDCAE in the pacemaking site from 1.2 ± 0.1 to 4.8 ± 0.3 AU/s (P<0.001). The treatment of VK-II-36 (30 μmol/L) did not inhibit caffeine-induced shifting of pacemaking site, sinus rate increase (152.7 ± 7.7 vs. 158.0 ± 5.0 bpm, P=0.59, Figure 5C), or LDCAE augmentation (4.2 ± 0.3 vs. 4.8 ± 0.3 AU/s, P=0.21, Figure 5D).

Discussion

The main findings of the present study are as follows. 1) A carvedilol analogue VK-II-36, a SOICR inhibitor, dose-dependently decreased sinus rate as with ryanodine treatment, and reduced LDCAE in the pacemaking site. 2) ISO induced an upward shift of the pacemaking site, coincident with the appearance of robust LDCAE. Sinus rate increased dose-dependently. Treatment with VK-II-36 reduced the ISO-induced effects on SAN function. 3) Caffeine also induced upward shift of the pacemaking site, coincident with the appearance of robust LDCAE, and increased sinus rate. Interestingly, the effects of caffeine were not inhibited by VK-II-36 treatment. These findings support the importance of spontaneous SR Ca^{2+} release (SOICR) for the pacemaker function in the intact canine SAN, especially during β-adrenergic stimulation.

This is the first report demonstrating that SOICR is involved in the increase of sinus rate, the shift of pacemaking site in SAN, and the augmentation of LDCAE at the pacemaking site during β-adrenergic stimulation. It has still been discussed whether Ca^{2+} clock or membrane clock plays a dominant role in SAN automaticity. Vinogradova et al. [4] reported that positive chronotropic effect of β-adrenergic stimulation is the result of increase in the Ca^{2+} transient caused by β-adrenergic stimulation, because they observed that the chronotropic effect in isolated SAN cells from rabbit is abolished or greatly reduced after the suppression of the Ca^{2+} transient by ryanodine. On the other hand, Honjo et al. [26] reported that SR Ca^{2+} release does not play a dominant role in pacemaker function in SAN, because ryanodine, which disables the SR Ca^{2+} release channel, did not abolish spontaneous activity in SAN. Our laboratory reported that ryanodine prevented ISO-induced LDCAE and blunted sinus rate acceleration, and that I_f blockade with ZD 7288 modestly blunted but did not prevent LDCAE or sinus rate acceleration by ISO in the intact canine dog [16]. Furthermore, the present study demonstrates that spontaneous SR Ca^{2+} release inhibition via SOICR blockade by VK-II-36 prevented ISO-induced upward shift of the pacemaking site in SAN and augmentation of LDCAE, and blunted sinus rate acceleration, which is similar to the effect of ryanodine. Our results suggest that sinus rate may be partly controlled by Ca^{2+} clock via SOICR during β-adrenergic stimulation. We demonstrated that SR Ca^{2+} release inhibition by ryanodine suppressed sinus rate both under basal condition and during β-adrenergic stimulation. SOICR inhibition by VK-II-36 also significantly decreased sinus rate with LDCAE reduction which indicates inhibition of spontaneous SR Ca^{2+} release. These

results suggest that SOICR is an important factor for SAN automaticity, especially during β-adrenergic stimulation.

VK-II-36 treatment shifted the pacemaking site to downward both under baseline condition and during β-adrenergic stimulation. It suggests that VK-II-36 is more effective in the superior SAN than the inferior SAN. The mechanism may be explained by the heterogeneity of SAN [27]. The key protein regulator of Ca uptake is phospholamban (PLB), which inhibits SERCA2a in its dephosphorylated state. β-adrenergic stimulation phosphorylates PLB, relieving its inhibition of SERCA2a and increasing Ca^{2+} uptake. As a result, spontaneous SR Ca^{2+} release occurs as a result of activation of the RyR channel by SR luminal Ca^{2+}. We previously reported that the SERCA2a/PLB molar ratio at SAN sites was significantly lower than at RA sites, and not significant but tends to be lower in superior SAN than in inferior SAN in canine hearts [16]. These may be involved in the heterogeneous effect to SAN function by SOICR inhibition.

β-adrenergic stimulation increases spontaneous SR Ca^{2+} release. Under conditions in which the SR Ca^{2+} content is abruptly increased, such as exercise, emotional stress, or on the infusion of cathecholamines, β-adrenergic receptors are activated, leading to activation of PKA, which in turn phosphorylates the L-type Ca^{2+} channel and PLB. This PKA phosphorylation increases both Ca^{2+} influx and SR Ca^{2+} uptake, resulting in an abrupt increase on SR free Ca^{2+}. The resulting SR Ca^{2+} spillover can activate the Na/Ca^{2+} exchanger, leading to increase of sinus rate. VK-II-36 inhibits SOICR. However, the detailed mechanism of VK-II-36-induced effect has yet to be determined. Jiang et al. [12] reported that CPVT RyR mutations enhance SOICR by increasing the channel sensitivity to activation by luminal Ca^{2+}, and alterations in RyR function are likely to contribute to the reduced threshold for SOICR, resulting in the high incidence of DAD-associated ventricular tachycardia. Our results could suggest that VK-II-36 inhibits spontaneous SR Ca^{2+} release by increasing the threshold for luminal Ca^{2+} activation of RyR. VK-II-36 inhibited ISO-induced effects in canine intact SAN, but did not inhibit caffeine-induced effects. Kong et al. [28] reported that caffeine triggers Ca^{2+} release by reducing the threshold for luminal Ca^{2+} activation of RyR. The present results suggest that caffeine reduced the threshold for luminal Ca^{2+} activation elevated by VK-II-36 and might cancel out the VK-II-36-induced inhibitory effect on SR Ca^{2+} release. As a result of that, caffeine shifted the leading pacemaker site to upward in SAN and augmented LDCAE at the pacemaking site, and increased sinus rate despite VK-II-36 treatment.

Another carvedilol analogue, VK-II-86, is known to have no effects on heart rate in either wild type or R4496C-heterozygous mice [20]. In comparison, VK-II-36 significantly reduced sinus rate in canine hearts. The mechanisms by which VK-II-36 exhibits differential effects in mice and canine SA nodes are unclear. One possibility is that the mice sinus node, which operates at an intrinsic rate many times faster than that of the canine sinus node, does not depend on the calcium release from the ryanodine receptor for heart rate control. However, such a view is not congruent with experimental results showing that calcium homeostasis in mouse SA node also plays an active role in heart rate acceleration (29). Alternatively, the ryanodine receptor in the mice sinus nodes may be less sensitive to VK

compound than the ryanodine receptors in canine sinus node. Further studies are required to test these hypotheses.

Limitations

There are several limitations in the present study. Firstly, we did not directly measure the SR Ca^{2+} release. It is therefore possible that some components of LDCAE might have originated from the membrane Ca^{2+} currents. However, we think LDCAE was originated from the SR Ca^{2+} release, because LDCAE was suppressed by VK-II-36 that reduced SR function via SOICR blocking effect. Secondly, VK-II-36 agent is a carvedilol analog that blocks SOICR but does not block β receptor. However, the other effects cannot be excluded. Yokoyama et al. [30] reported carvedilol inhibits the Ca current, delayed rectifier K^+ current and hyperpolarization-activated inward current in the SAN cells with voltage clamp experiments. VK-II-36 may have the same effects, and cause a suppression of spontaneous pacemaker activity by the inhibition of various currents. Thirdly, the blebbistatin used for inhibiting cardiac contractility is an excitation-contraction uncoupling drug, and may influence the intracellular Ca dynamics. Fedorov et al. [24] reported that blebbistatin increased resting fluorescence by 39%. We cannot rule out the possibility that the blebbistatin may influence our data and the interpretation of the results. Fourthly, we reported that LDCAE were observed in only 4 of 25 preparations in the previous paper [16]. The exact reason why the appearance of LDCAE in this study is different from the previous paper is unclear. It might be attributed to improved experimental technique and data quality, allowing better detection of small LDCAE in this study.

Conclusions

In conclusion, SOICR is important for SAN automaticity. Sinus rate might be partly controlled by Ca^{2+} clock via SOICR during β-adrenergic stimulation. Spatial heterogeneity of SOICR may be a key in determining the site of dominant pacemaker. Further studies on SOICR may lead to the development of novel treatments for SAN dysfunction.

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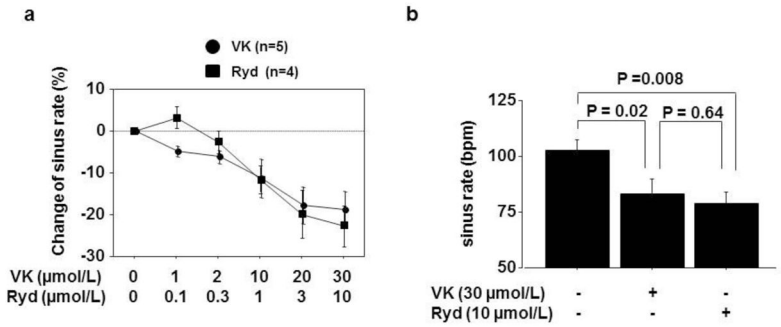

Figure 1. The effect of SR Ca release inhibitor on intact SAN

a, Changes of sinus rate by VK-II-36 or ryanodine infusion. b, Sinus rate at 30 μmol/L VK-II-36 or 10 μmol/L ryanodine infusion. VK = VK-II-36; Ryd = ryanodine.

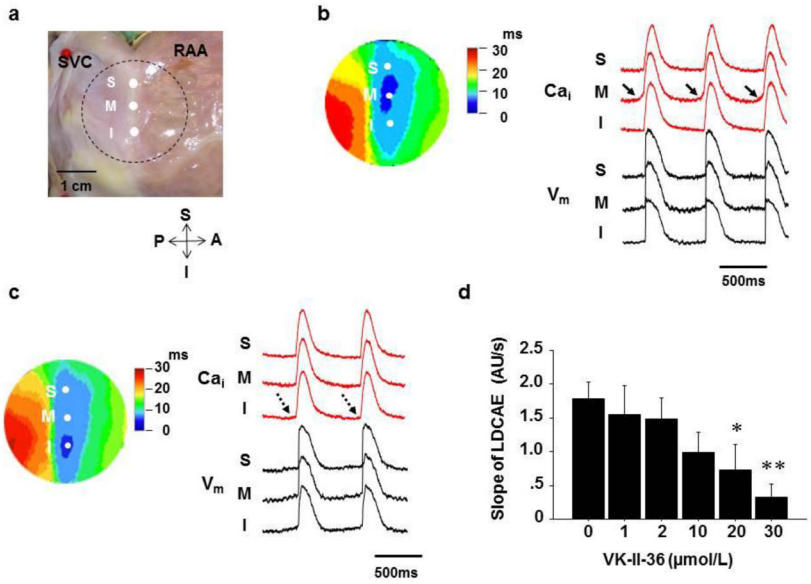

Figure 2. The change of activation pattern of SAN by SR Ca release inhibitor, VK-II-36

a, Photo of the isolated RA preparation showing the SAN and surrounding RA. b, Activation pattern of the SAN and surrounding RA under basal condition. The color picture shows isochronal map of V_m propagation. The Ca_i (red) and V_m (black) recordings from the superior (S), middle (M), and inferior (I) SAN are presented. Arrows point to late diastolic Ca_i elevation (LDCAE). c, Activation pattern of SAN and surrounding RA during VK-II-36 infusion of 20 μmol/L. Dotted arrows point to no LDCAE. d, Effect of VK-II-36 (0 to 30 μmol/L) on the slope of LDCAE. SVC = superior vena cava; RAA = right atrial appendage.

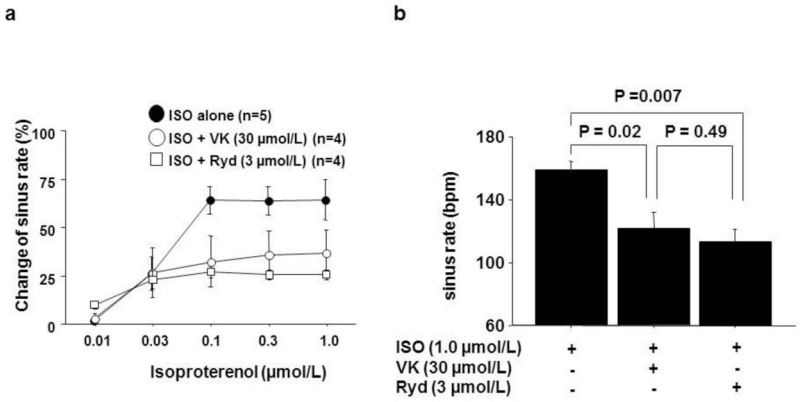

Figure 3. The effect of SR Ca release inhibitor to isoproterenol-induced SAN function change

a, Effects of VK-II-36 or ryanodine on isoproterenol-induced sinus rate change. b, Sinus rate at 1.0 μmol/L isoproterenol infusion with VK-II-36 (30 μmol/L) or ryanodine (3 μmol/L). ISO = isoproterenol; VK = VK-II-36; Ryd = ryanodine.

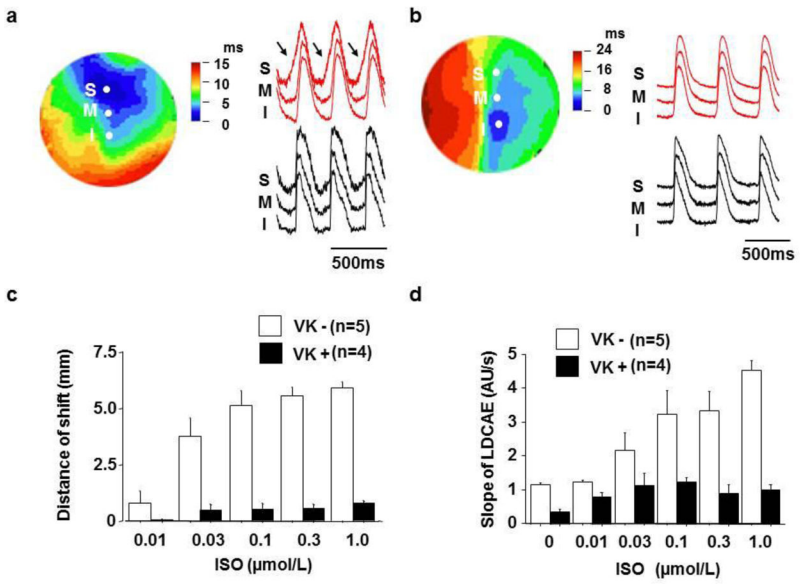

Figure 4. Effect of isoproterenol on activation pattern of SAN during VK-II-36 treatment

a, Isoproterenol (1.0 μmol/L) -induced activation pattern of SAN. Color picture shows isochronal map of V_m. The Ca_i (red) and V_m (black) recordings from the superior (S), middle (M), and inferior (I) SAN are presented. Arrows point to late diastolic Ca_i elevation (LDCAE). b, Effect of VK-II-36 (30 μmol/L) treatment to Isoproterenol (1.0 μmol/L) -induced activation pattern of SAN. c, Distance of shift to upward by isoproterenol infusion with VK-II-36 (30 μmol/L) treatment or not. d, Slope of LDCAE by isoproterenol infusion with VK-II-36 (30 μmol/L) or not. ISO = isoproterenol; VK = VK-II-36.

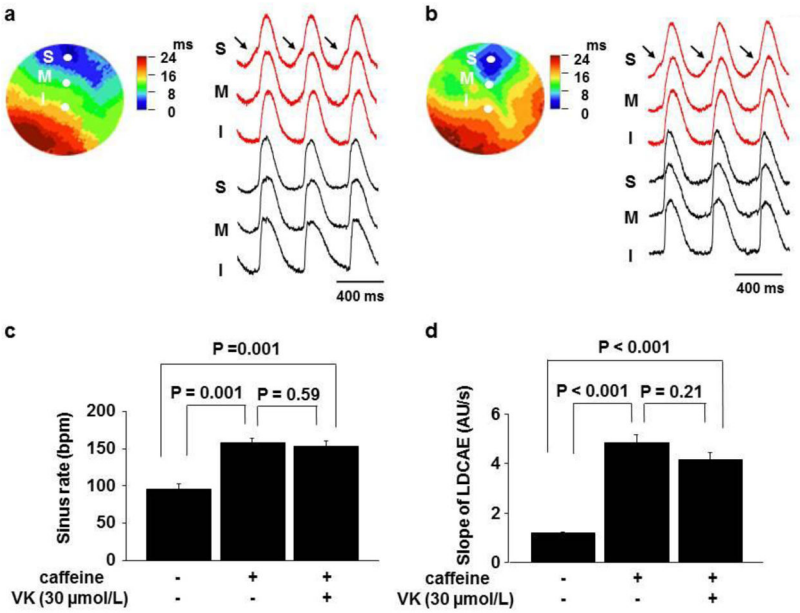

Figure 5. Effect of caffeine on activation pattern of SAN during VK-II-36 treatment

a, Caffeine (20 mmol/L, 2-mL bolus given within 1 sec)-induced activation pattern of SAN. Color picture shows isochronal map of Vm. The Cai (red) and Vm (black) recordings from the superior (S), middle (M), and inferior (I) SAN are presented. Arrows point to late diastolic Cai elevation (LDCAE). b, Effect of VK-II-36 (30 µmol/L) treatment to Caffeine-induced activation pattern of SAN. Arrows point to LDCAE. c, Sinus rate change by caffeine infusion with VK-II-36 (30 µmol/L) treatment or not. d, Slope of LDCAE by caffeine infusion with VK-II-36 (30 µmol/L) treatment or not. VK = VK-II-36.